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Isolation and characterization of a gene encoding a G-protein α subunit from *Schizosaccharomyces pombe*: Involvement in mating and sporulation pathways

(guanine nucleotide-binding proteins/fission yeast/signal transduction/sterility)

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ABSTRACT **The *gpa1* gene of *Schizosaccharomyces pombe*, which encodes a protein homologous with the α subunits of mammalian guanine nucleotide-binding proteins (G proteins), was isolated by cross-hybridization using rat $G_i1\alpha$ and $G_x\alpha$ cDNA. The deduced amino acid sequence was about 37% identical with rat $G_i1\alpha$ and $G_x\alpha$ proteins and contained three conserved motifs commonly found in all GTP-binding proteins. Disruption of *gpa1* was not lethal but conferred sterility and sporulation deficiency on *Sch. pombe* cells. Thus, the gene is essential for the sexual development and is probably coupled to mating-factor receptors. In contrast to *Saccharomyces cerevisiae GPA1*, which plays a negative role in mating-factor signal transduction, *Sch. pombe gpa1*$^+$ apparently has a positive function. A *gpa1* transcript of 2.2 kilobases was detected in vegetatively growing cells. A 1.6-kilobase *gpa1* transcript appeared in addition to the 2.2-kilobase transcript when cells were derepressed for mating or meiosis.**

Heterotrimeric guanine nucleotide-binding regulatory proteins (G proteins) are involved in a variety of receptor-mediated signaling systems. They are widely distributed among eukaryotic organisms and their structures are highly conserved (see ref. 1 for a review). The cloning of cDNAs and genomic DNAs for G-protein α subunits has recently revealed that at least nine genes code for α subunits of G proteins in mammalian cells (see ref. 2 for a review). These genes encode proteins whose structures are closely related but distinct. Furthermore, at least two of these genes, $G_s1\alpha$ and $G_o\alpha$, generate several subtypes by alternative splicing (3, 4).

G proteins are also found in lower organisms such as *Drosophila, Dictyostelium*, and *Saccharomyces cerevisiae*. We have isolated two genes coding for G-protein α subunits from *S. cerevisiae* and designated them *GPA1* and *GPA2* (5, 6). Subsequent studies have revealed that *GPA1* is involved in the mating-factor signal transduction in *S. cerevisiae* (7–9). It is coupled to the *STE2* and *STE3* products, which are the cell-surface receptors for α and a factors, respectively, and elicits the signals for mating and G_1 arrest (see refs. 10 and 11 for reviews). *GPA1* is expressed only in haploid cells and its disruption results in a haploid-specific lethal phenotype (7, 8). On the other hand, *GPA2* is expressed in both haploid and diploid cells, and it seems to play a role in the cAMP pathway of *S. cerevisiae* (6).

The fission yeast *Schizosaccharomyces pombe* is another genetically tractable microorganism that has been widely utilized as a model eukaryotic cell. *Sch. pombe* cells initiate sexual development in response to nitrogen starvation (12), and cells of the opposite mating types communicate through mating factors (13, 14). A putative mating-factor receptor that has homology to *S. cerevisiae STE3* has been identified (K. Tanaka, Y. Imai, and M.Y., unpublished work). Thus, it is conceivable that the signals from the mating factors of *Sch. pombe* are also transmitted through a G protein into the effector(s).

In this paper, we describe a gene coding for a G-protein α subunit (designated *gpa1*) from *Sch. pombe* that has been isolated by hybridization with mammalian G-protein α subunit cDNAs.¶ The nucleotide and the deduced amino acid sequences are homologous to those of mammalian as well as *S. cerevisiae* G-protein α subunit cDNAs. Genetic studies indicate that *Sch. pombe gpa1* is not essential for vegetative growth but is involved in the developmental pathway for mating and sporulation.

MATERIALS AND METHODS

Strains, Media, and Genetic Methods. Table 1 lists the *Sch. pombe* strains used. Complete medium YPD and minimal medium SD (15) were employed for routine cell culture. Minimal medium PM (16) and its derivative PM-N, which lacks NH_4Cl, were also used. Malt extract agar (MEA) plates were used to induce mating and sporulation (17). General genetic methods were as described by Gutz *et al.* (17). *Sch. pombe* cells were transformed by the lithium method originally described for *S. cerevisiae* (18) and then modified for *Sch. pombe* (19). Gene replacement on the *Sch. pombe* genome was done essentially as described (20, 21).

Southern and Northern Blot Analyses. *Sch. pombe* DNA was prepared (22) and Southern blot analysis was carried out under low- and high-stringency conditions as described (5), except that *Escherichia coli* tRNA (100 μg/ml) was substituted for heat-denatured calf thymus DNA.

Total cellular RNA was extracted (23) either from logarithmically growing *Sch. pombe* cells (5×10^6 per ml) in PM medium or from cells shifted to PM-N medium and incubated for 4 hr. Denaturation of RNA, gel electrophoresis, blotting, and hybridization were as described (24).

Nucleotide Sequence Determination. Nucleotide sequences were determined by the dideoxynucleotide chain-termination method (25).

Other Methods. *In vitro* site-directed mutagenesis of *Sch. pombe gpa1* was performed as described (26). Other procedures were as described by Sambrook *et al.* (27).

RESULTS

Cloning of the *gpa1* Gene. *Sch. pombe* DNA was digested with several restriction endonucleases and subjected to

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¶The sequence reported in this paper has been deposited in the GenBank data base (accession no. M64286).

Table 1. *Sch. pombe* strains used

Strain	Genotype
L972	h^- wild type
L975	h^+ wild type
JY3	h^{90} wild type
JY741	h^- *ade6-M216 leu1 ura4-D18*
JY746	h^+ *ade6-M210 leu1 ura4-D18*
JY765	h^+/h^- *ade6-M210/ade6-M216 leu1/leu1 ura4-D18/ura4-D18*
JY879	h^{90} *ade6-M210 leu1 ura4-D18*
JY919	h^+/h^- *ade6-M210/ade6-M216*
JZ451	h^+ *ade6-M210 leu1 ura4-D18 gpa1::ura4*$^+$
JZ452	h^- *ade6-M216 leu1 ura4-D18 gpa1::ura4*$^+$
JZ453	h^{90} *ade6-M210 leu1 ura4-D18 gpa1::ura4*$^+$
JZ454	h^+/h^- *ade6-M210/ade6-M216 leu1/leu1 ura4-D18/ura4-D18 gpa1::ura4*$^+$/+
JZ455	h^+/h^- *ade6-M210/ade6-M216 leu1/leu1 ura4-D18/ura4-D18 gpa1::ura4*$^+$/*gpa1::ura4*$^+$

Southern blot analysis under low-stringency conditions. The probe for hybridization was either the C-terminal 0.7-kilobase (kb) *Pst* I fragment of rat $G_i1\alpha$ cDNA (28) or the 1.0-kb *Nco* I–*Bgl* II fragment that covers almost the entire length of rat $G_x\alpha$ cDNA (29). Both probes hybridized with a single band of the same size in each digest (data not shown). To clone this sequence, we screened a *Sch. pombe* genomic library made of size-fractionated *Sau*3A1 partial digests of *Sch. pombe* DNA inserted in pDB248′ (30). One positive clone (621B2) was finally obtained. The plasmid recovered from 621B2 carried a 0.4-kb segment that could encode an amino acid sequence significantly homologous to the C-terminal region of G-protein α subunits. Genomic Southern blot analysis using the 0.4-kb segment as a probe detected single bands of 3.0 kb and 4.2 kb, respectively, in *Pst* I and *Eco*RI digests. Both fragments, which were cloned, contained the full coding sequence of *gpa1* (Fig. 1). A composite segment

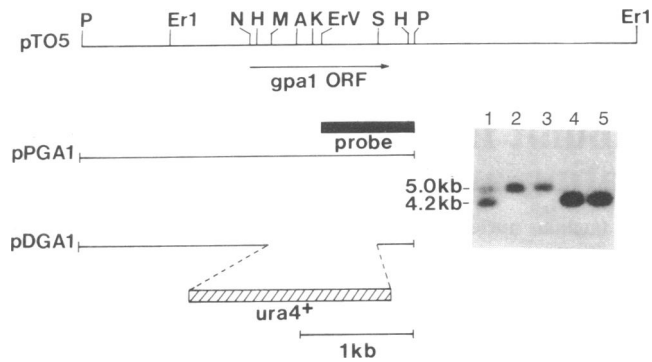

FIG. 1. Restriction map of the *gpa1* gene, relevant plasmids, and Southern blot analysis of the disruption of the chromosomal *gpa1* gene. The 5.0-kb region cloned in the plasmid pTO5 is shown (see text for details). The arrow indicates the *gpa1* open reading frame (ORF). Recognition sites for restriction endonucleases: A, *Acc* III; Er1, *Eco*RI; ErV, *Eco*RV; H, *Hin*dIII; K, *Kpn* I; M, *Mlu* I; N, *Nco* I; P, *Pst* I; S, *Spe* I. Plasmid pPGA1 carries the 3.0-kb *Pst* I fragment derived from pTO5, which is sufficient to complement the *gpa1* deficiency (data not shown). pDGA1 was constructed from pPGA1 and was used to perform gene disruption of an allele of *gpa1* in a diploid cell, JY765. *Inset* shows Southern blot analysis of DNAs of a diploid, JZ454 (lane 1), and a set of haploid progenies derived from it (lanes 2–5). DNAs were digested with *Eco*RI and probed with the ^{32}P-labeled *Eco*RV–*Pst* I fragment of pTO5 (indicated as a solid bar). The 4.2-kb band corresponds to the intact *gpa1* allele and the 5.0-kb band indicates the disrupted allele.

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ATAATAGTATTCTTTTGGATCTCTTGGAATTATCTGGCAATTGGGTTTTTTGCTTATAGTTGGGTCCACTTCACAAATATTTGAGATTTAACGACATCTATTATTTCTTTTTTAATCCAA
AAATCCTTATTTATCACCTTTTCTGCTGTTTTTACAGAAAATTGCGGAGATTAACTTTCTTTGTCTCGACTTTGAATTATCTCCTTGTGGGGATTTGGAATCTGTAGCTGCCTTTTCACC

  1: ATG GGA TGC ATG TCG AGT AAA TAC GCT GAT ACA TCA GGA GGA GAA GTC ATT CAA AAG AAG CTT TCA GAT ACG CAA ACC TCA AAC AGC TCT
  1: Met Gly Cys Met Ser Ser Lys Tyr Ala Asp Thr Ser Gly Gly Glu Val Ile Gln Lys Lys Leu Ser Asp Thr Gln Thr Ser Asn Ser Ser

 91: ACA ACT GGA AGT CAA AAC GCT CGA GTT CCA GTC CTT GAA AAC TGG CTT AAT ATC GTC CTG CGT GGA AAA CCA CAA AAT GTG GAA AGT TCT
 31: Thr Thr Gly Ser Gln Asn Ala Arg Val Pro Val Leu Glu Asn Trp Leu Asn Ile Val Leu Arg Gly Lys Pro Gln Asn Val Glu Ser Ser

181: GGA GTA CGC GTA AAA GGA AAT TCT ACT TCA GGT GGA AAT GAC ATT AAA GTT TTG CTC TTA GGC GCC GGT GAT AGT GGG AAA ACG ACC ATT
 61: Gly Val Arg Val Lys Gly Asn Ser Thr Ser Gly Gly Asn Asp Ile Lys Val Leu Leu Leu Gly Ala Gly Asp Ser Gly Lys Thr Thr Ile

271: ATG AAG CAG ATG AGA TTA TTG TAT AGC CCC GGT TTT AGT CAA GTA GTT AGA AAG CAG TAT CGA GTG ATG ATT TTT GAA AAT ATC ATC TCC
 91: Met Lys Gln Met Arg Leu Leu Tyr Ser Pro Gly Phe Ser Gln Val Val Arg Lys Gln Tyr Arg Val Met Ile Phe Glu Asn Ile Ile Ser

361: TCT CTA TGT CTT CTT CTT GAA GCT ATG GAT AAT AGT AAT GTC TCT TTA CTT CCG GAA AAT GAG AAG TAT CGG GCA GTT ATC CTA AGA AAA
121: Ser Leu Cys Leu Leu Leu Glu Ala Met Asp Asn Ser Asn Val Ser Leu Leu Pro Glu Asn Glu Lys Tyr Arg Ala Val Ile Leu Arg Lys

451: CAC ACT TCT CAA CCC AAT GAG CCA TTT TCT CCA GAA ATA TAT GAA GCT GTT CAT GCC TTG ACA TTG GAT ACC AAA CTT CGT ACG GTG CAA
151: His Thr Ser Gln Pro Asn Glu Pro Phe Ser Pro Glu Ile Tyr Glu Ala Val His Ala Leu Thr Leu Asp Thr Lys Leu Arg Thr Val Gln

541: AGT TGT GGT ACC AAC CTC TCT TTG TTA GAC AAT TTT TAT TAC TAT CAA GAT CAC ATT GAT CGA ATT TTT GAC CCA CAA TAT ATA CCT TCT
181: Ser Cys Gly Thr Asn Leu Ser Leu Leu Asp Asn Phe Tyr Tyr Tyr Gln Asp His Ile Asp Arg Ile Phe Asp Pro Gln Tyr Ile Pro Ser

631: GAT CAA GAT ATC CTT CAC TGT CGT ATC AAG ACG ACC GGT ATA TCA GAA GAA ACA TTT CTG TTA AAT CGT CAT CAT TAC CGA TTT TTT GAT
211: Asp Gln Asp Ile Leu His Cys Arg Ile Lys Thr Thr Gly Ile Ser Glu Glu Thr Phe Leu Leu Asn Arg His His Tyr Arg Phe Phe Asp

721: GTA GGA GGA CAG AGA TCA GAG CGC AGA AAA TGG ATT CAT TGC TTT GAA AAT GTC ACT GCA TTG TTG TTT CTC GTT TCT TTG GCA GGT TAC
241: Val Gly Gly Gln Arg Ser Glu Arg Arg Lys Trp Ile His Cys Phe Glu Asn Val Thr Ala Leu Leu Phe Leu Val Ser Leu Ala Gly Tyr

811: GAT CAA TGC CTT GTA GAG GAC AAT TCA GGA AAT CAG ATG CAG GAG GCG TTA TTA TTA TGG GAT TCC ATA TGT AAC TCT AGC TGG TTT TCA
271: Asp Gln Cys Leu Val Glu Asp Asn Ser Gly Asn Gln Met Gln Glu Ala Leu Leu Leu Trp Asp Ser Ile Cys Asn Ser Ser Trp Phe Ser

901: GAA TCA GCA ATG ATA CTT TTT CTA AAT AAA CTT GAT TTA TTT AAA AGA AAA GGT TCA CAT TTC CCC ATC CAG AAG CAT TTT CCT GAT TAC
301: Glu Ser Ala Met Ile Leu Phe Leu Asn Lys Leu Asp Leu Phe Lys Arg Lys Gly Ser His Phe Pro Ile Gln Lys His Phe Pro Asp Tyr

991: CAA GAA GTT GGT TCA ACA CCA ACA TTC GTA CAA ACT CAA TGC CCT CTT GCC GAC AAC GCA GTT CGA AGC GGT ATG TAT TAC TTT TAC TTA
331: Gln Glu Val Gly Ser Thr Pro Thr Phe Val Gln Thr Gln Cys Pro Leu Ala Asp Asn Ala Val Arg Ser Gly Met Tyr Tyr Phe Tyr Leu

1081: AAG TTT GAA AGT CTT AAT CGC ATC GCT TCT CGT AGT TGC TAT TGC CAT TTT ACC ACA GCT ACA GAC ACT AGT TTG CTC CAA AGG GTA ATG
361: Lys Phe Glu Ser Leu Asn Arg Ile Ala Ser Arg Ser Cys Tyr Cys His Phe Thr Thr Ala Thr Asp Thr Ser Leu Leu Gln Arg Val Met

1171: GTA TCC GTT CAA GAT ACG ATT ATG TCC AAC AAT CTA CAG TCA CTT ATG TTT TAG ATGAATTTTTCCTTAACTATCTTCATAATCTTTTCTGTCTCATTTCTC
391: Val Ser Val Gln Asp Thr Ile Met Ser Asn Asn Leu Gln Ser Leu Met Phe End

TACTTTCATTCGCACTCTAGGAAAAGCCTCTATACTTTTCCGTGAATGGTGTCTCTAGTTAATTTCCTTGCATTTTGTATGCACTTCATGTGTTATTTCTCCATCGAAACTGTTAATTAG
TGCATTCTAATTGTTTTATTTCATAGCTTCTTCGAAGCTTCGTGTAACTTATATTGTTTTAAATAGCTCAAAATTAACTGCAG
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FIG. 2. Nucleotide and predicted amino acid sequence of the *gpa1* gene. Numbering starts at the A of the deduced initiation codon.

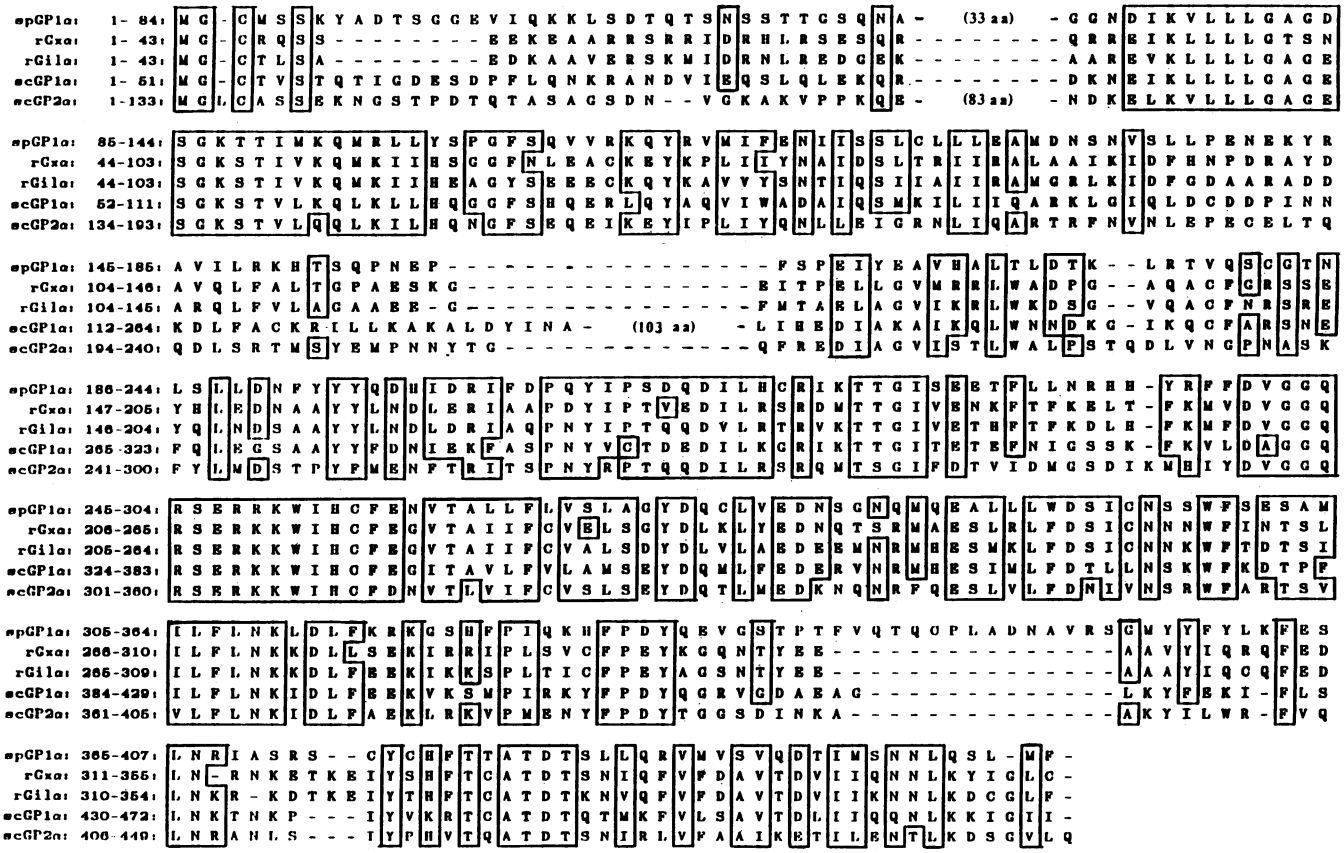

FIG. 3. Alignment of the predicted amino acid sequence of the *Sch. pombe* Gpa1 protein (spGP1α) with those of rat $G_i1\alpha$ (rGi1α; ref. 28) and $G_x\alpha$ (rGxα; ref. 29), and *S. cerevisiae* GP1α (scGP1α; ref. 5) and GP2α (scGP2α; ref. 6). Sets of four or more identical or conservative residues with respect to *Sch. pombe* Gpa1 are enclosed in the boxes. Amino acid residues are represented by the standard one-letter symbols. Dashes indicate gaps inserted to optimize homology (aa, amino acids). Conservative amino acid substitutions are grouped as follows according to Dayhoff classification (31); C; S, T, P, A, and G; N, D, E, and Q; H, R, and K; M, I, L, and V; F, Y, and W.

comprising the two fragments was cloned in pUC118 to give pTO5 (Fig. 1).

Nucleotide Sequence of *gpa1*. The nucleotide sequence of pTO5 revealed an open reading frame that encodes a protein of 407 amino acids with a calculated M_r of 46,254 (Fig. 2). No introns were evident in the nucleotide sequence. Comparison of the deduced amino acid sequence of the *gpa1*-encoded protein (Gpa1) with those of the known G-protein α subunits revealed the amino acid identity shown in Fig. 3. In the Gα family, mammalian $G_i1\alpha$ and $G_x\alpha$ were the most similar to Gpa1 (about 37%). *S. cerevisiae* GPA1 and GPA2 were less homologous to *Sch. pombe* Gpa1 (about 34%). Amino acid sequences unique to *Sch. pombe* are evident; one in the N-terminal region and one in the C-terminal region (Fig. 3). Three regions called P, G′, and G, which are highly conserved in all Gα proteins (2), are detected in the amino acid sequence of *Sch. pombe* Gpa1.

Disruption of *gpa1*. A disrupted allele of *gpa1* was constructed in pPGA1, a pUC119-based plasmid carrying the 3.0-kb *Pst* I fragment that covers the whole *gpa1* open reading frame (Fig. 1). A 1.0-kb *Mlu* I–*Spe* I fragment was replaced by a 1.8-kb *Sch. pombe ura4*$^+$ cassette, giving the plasmid pDGA1 (Fig. 1). This plasmid was cut with *Pst* I to obtain linear DNA molecules that were introduced into a *Sch. pombe* diploid strain, JY765 (*ura4*$^-$/*ura4*$^-$). Stable Ura$^+$ transformants were selected. Structures at the *gpa1* locus in the transformants were probed with the *Eco*RV–*Pst* I fragment (Fig. 1) in Southern blots. A typical disrupted allele in JZ454 is shown in Fig. 1. JZ454 apparently carried one wild-type allele and one disrupted allele of *gpa1*. Sporulation was induced in JZ454 and the progeny spores were dissected. In most tetrads, all four spores were viable, and they segregated 2 Ura$^+$:2 Ura$^-$. Southern blots showed that the wild-type and disrupted alleles also segregated 2:2 (Fig. 1, lanes 2–5). Moreover, cells carrying the disrupted allele were always Ura$^+$. Not only is disruption of *gpa1* not lethal, but the disruptants grew at the same rate as wild-type strains in all of the media examined.

Sterility and Sporulation Deficiency of *gpa1* Disruptants. Although no defect in cell growth was apparent, *gpa1* disruptants were found to be completely sterile (Table 2). When either partner (*h*$^+$ or *h*$^-$) was *gpa1*$^-$, mating did not occur (Fig. 4). Successful mating and subsequent sporulation can be observed only in Fig. 4*D*. We constructed an *h*$^+$/*h*$^-$ *gpa1*$^-$/*gpa1*$^-$ diploid strain, JZ455, by protoplast fusion (32). This strain could not sporulate (Table 2). Thus *gpa1* function appears to be essential for both mating and sporulation. To

Table 2. Effects of *gpa1* disruption on sexual development of *Sch. pombe*

Strain	Genotype	Mating efficiency*, %	Sporulation efficiency†, %
JY879	*h*^{90} *gpa1*$^+$	73	
JZ453	*h*^{90} *gpa1*$^-$	<0.03	
JY765	*h*$^+$/*h*$^-$ *gpa1*$^+$/*gpa1*$^+$		83
JY455	*h*$^+$/*h*$^-$ *gpa1*$^-$/*gpa1*$^-$		<0.03

*Cells were grown to 3 × 10^6 cells per ml at 30°C in PM medium and shifted to PM-N medium containing only 1% glucose. After a 24-hr incubation, the numbers of zygotes, asci, free spores, and unmated cells were determined microscopically.

†The same procedure was followed as above, except that asci, free spores, and unsporulated cells were counted.

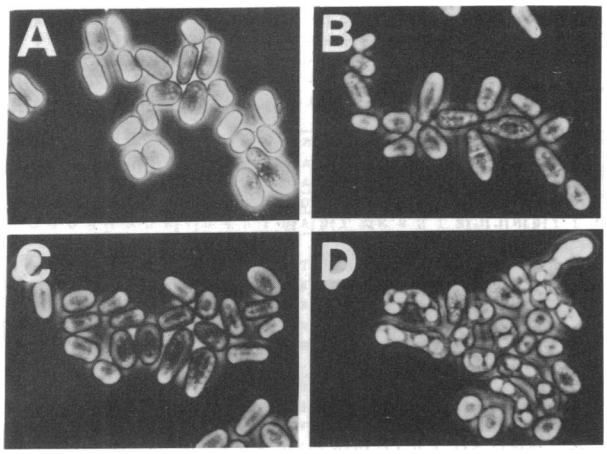

FIG. 4. Sterility of *gpa1* disruptants. Cells of the opposite mating types were mixed and plated on MEA medium. After incubation for 18 hr at 30°C, photographs were taken under phase-contrast microscopy. (*A*) JZ451 (h^+ *gpa1*$^-$) × JZ452 (h^- *gpa1*$^-$). (*B*) JY746 (h^+ *gpa1*$^+$) × JZ452 (h^- *gpa1*$^-$). (*C*) JZ451 (h^+ *gpa1*$^-$) × JY741 (h^- *gpa1*$^+$). (*D*) JY746 (h^+ *gpa1*$^+$) × JY741 (h^- *gpa1*$^+$).

investigate whether Gpa1 protein is involved in recognition of mating factors, a mutation (Gln-244 to Leu) that is supposed to activate G-protein α subunits (33, 34) was introduced into *gpa1* by *in vitro* mutagenesis. Heterothallic strains carrying this *gpa1-L244* allele, whether h^+ or h^-, extended conjugation tubes in the absence of the mating partner and under nitrogen starvation (Fig. 5). This suggests that once Gpa1 is activated, cells can exhibit a mating response without receiving the mating factors, and that Gpa1 is likely to be coupled with mating factor receptors.

***gpa1* Transcription.** Transcription of *gpa1* was detected in RNA blots of total cellular RNA (Fig. 6). Growing cells contained a transcript of 2.2 kb, independent of their mating type. Heterothallic haploid (h^+ and h^-), homothallic haploid (h^{90}), and heterozygous diploid (h^+/h^-) strains all produced this transcript. Little change was observed in transcription pattern when h^+ or h^- cells were starved for nitrogen. However, when h^{90} or h^+/h^- cells were starved for nitrogen, a 3- to 4-fold increase in the amount of the 2.2-kb transcript

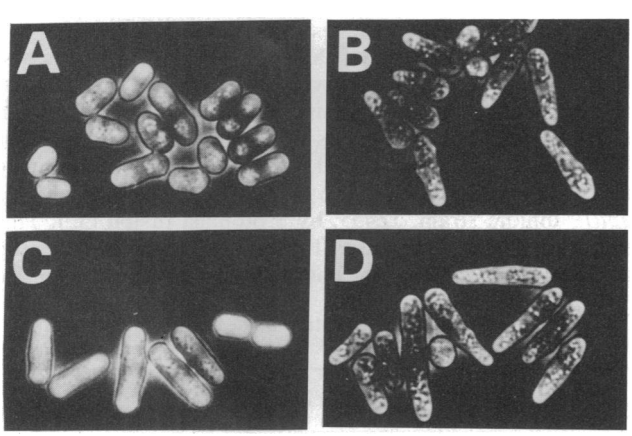

FIG. 5. Spontaneous extension of conjugation tubes displayed by heterothallic *Sch. pombe* cells carrying activated Gpa1 protein under nitrogen starvation. Two *gpa1* disruptants, JZ451 (h^+) and JZ452 (h^-) were transformed with pDB(gpa1QL), a plasmid which is based on pDB248′ (30) and carries the activated *gpa1-L244* allele. Cells were grown to 3 × 10^6 per ml in PM medium at 30°C, then shifted to PM-N medium and incubated for 16 hr. (*A*) JZ451 with pDB(gpa1QL), growing. (*B*) The same strain, starved for nitrogen. (*C*) JZ452 with pDB(gpa1QL), growing. (*D*) The same strain, starved for nitrogen. Extension of conjugation tubes is evident in *B* and *D*.

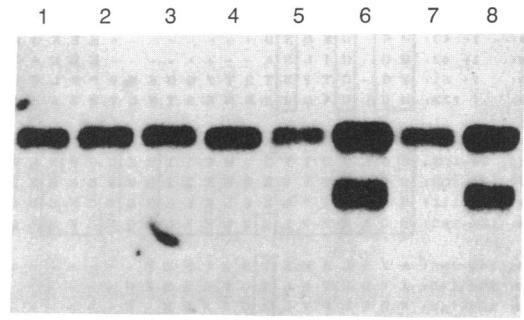

FIG. 6. RNA blot analysis of *gpa1* transcripts. *Sch. pombe* strains were grown to 5 × 10^6 cells per ml in PM medium at 30°C. Half of the culture was harvested and the remainder was starved for nitrogen for 4 hr in PM-N medium. Total cellular RNA was prepared and subjected to RNA blot analysis. Each lane received 5 μg of RNA. The hybridization probe was the ^{32}P-labeled 0.96-kb *Mlu* I–*Spe* I fragment of pTO5 (see Fig. 1). Lanes 1 and 2, L975 (h^+); lanes 3 and 4, L972 (h^-); lanes 5 and 6, JY3 (h^{90}); lanes 7 and 8, JY919 (h^+/h^-). Odd-numbered lanes, logarithmically growing cells; even-numbered lanes, cells starved for nitrogen.

was observed and, more strikingly, a new transcript of 1.6 kb emerged. Nuclease S1 mapping analysis indicated that the shorter transcript was transcribed from the same *gpa1* gene but that the transcription initiation site was about 600 nucleotides downstream from that of the 2.2-kb transcript (data not shown).

DISCUSSION

The yeast Gα proteins are considerably larger than their mammalian counterparts; proteins encoded by *S. cerevisiae GPA1*, *S. cerevisiae GPA2*, and *Sch. pombe gpa1* are 472, 449, and 407 amino acids long, respectively, whereas mammalian $G_s1\alpha$ and $G_{i2}\alpha$ are, respectively, 394 and 355 amino acids in length. The *S. cerevisiae* GPA1 and GPA2 proteins are, respectively, 110 amino acids (residues 126–235) and 83 amino acids (residues 37–119) longer, with their insertions near the N terminus. Alignment of the *Sch. pombe* Gpa1 sequence with other Gα proteins revealed short additional sequences in *Sch. pombe* in both N- and C-terminal regions (residues 38–70 and 339–353). Like *S. cerevisiae* GPA1 and GPA2 proteins, *Sch. pombe* Gpa1 did not contain the cysteine residue near the C terminus that has been identified as the site for ADP-ribosylation by pertussis toxin in certain groups of mammalian Gα proteins. However, the arginine residue that is modified by cholera toxin-catalyzed ADP-ribosylation of mammalian $G_s\alpha$, Arg-201, is conserved in *Sch. pombe* Gpa1 (Arg-218) as well as *S. cerevisiae* GPA1 (Arg-297) and GPA2 (Arg-273).

Genetic studies with a disrupted *gpa1* gene indicated that *Sch. pombe gpa1* is required for mating and sporulation but not for vegetative growth (Fig. 4 and Table 2). Since the *S. cerevisiae GPA1* gene product is coupled with the mating factor receptors (products of *STE2* and *STE3*) and functions in mating-factor signal transduction (7–11), it is conceivable that *Sch. pombe* Gpa1 may also be involved in signaling by its mating factor. The results in Fig. 5 strongly suggest that this is the case. A gene for the mating-factor receptor in *Sch. pombe* has recently been isolated and would encode a protein having seven transmembrane helices like other Gα-coupled receptors (K. Tanaka, Y. Imai, and M.Y., unpublished work). We speculate that *Sch. pombe* Gpa1 is directly coupled with a mating-factor receptor.

An intriguing difference is observed between the functions of *Sch. pombe gpa1* and *S. cerevisiae GPA1*. In *S. cerevisiae*, disruption of *GPA1* was lethal in haploid cells, whereas the *gpa1*-disrupted *Sch. pombe* cells were viable but sterile. In *S. cerevisiae*, genetic data suggest that the α subunit (GPA1)

plays a negative role in signal transduction, whereas the β and γ subunits (STE4 and STE18) function as positive factors that transmit the signals downstream (7–9, 35–38). Therefore, disruption of *GPA1* activates the mating-factor signaling pathway and arrests the cell cycle in G_1 (7–9), inducing the genes essential for mating (39). In agreement with this view, loss of functions of either *STE4* or *STE18* caused sterility (35). In contrast, *Sch. pombe* Gpa1 appears to function as a positive factor that transmits the signal from the mating-factor receptors to downstream effector(s). In this respect, the mode of function of *Sch. pombe* Gpa1 is more like that of mammalian G-protein α subunits. The genes for *Sch. pombe* G-protein β and γ subunits have not been identified, nor their functions determined.

S. cerevisiae diploid cells defective in *GPA1* are viable and sporulation-proficient, suggesting that *S. cerevisiae GPA1* is not required in diploids. The function of *gpa1* is required for sporulation in *Sch. pombe*, which may be puzzling at first sight. However, the mating factor(s) may be necessary for induction of sporulation in *Sch. pombe*. The *map1* mutation causes h^+ mating type-specific sterility in *Sch. pombe*, and *map1/map1* diploid cells are deficient in sporulation (14). This sporulation deficiency can be rescued by a diffusible substance(s) from h^+ cells, which may be the mating factor itself (14). Further analysis of the *Sch. pombe* mating-factor pathway is needed to demonstrate unequivocally that mating factors are involved in both mating and sporulation.

Fig. 6 indicates that the transcription of *gpa1* is induced on nitrogen starvation in homothalic haploid (h^{90}) and heterozygous diploid (h^+/h^-) cells, and a new species of *gpa1* mRNA (1.6 kb) appears concomitantly with an increase of the preexisting 2.2-kb transcript. It is intriguing that the new transcript is induced only when cells are being derepressed for sexual development.

The function of adenylyl cyclase or cAMP differs considerably between *S. cerevisiae* and *Sch. pombe*. In *S. cerevisiae*, cAMP is required for the progression of the cell cycle (40), whereas *Sch. pombe* cells can grow in the complete absence of cAMP and adenylyl cyclase (41). The intracellular cAMP level plays an important role in the regulation of the initiation of sexual development in *Sch. pombe* (41). Thus, it is possible that Gpa1 could cause effects on mating and sporulation through regulation of adenylyl cyclase activity. However, this is unlikely because cells devoid of cAMP are sexually derepressed but do not bypass the requirement of mating factors, whereas cells carrying activated Gpa1 show a sexual response in the absence of mating factors. Conversely, *cyr1*$^-$ cells disregard the requirement of nitrogen starvation for sexual development (41) but *gpa1*$^-$ cells do not (Fig. 5).

Further analysis of Gpa1 function is highly relevant to elucidation of the function of the Ras homolog in *Sch. pombe*. From previous observations (42–44), we propose that the Ras protein regulates the sensitivity of the mating-factor recognition pathway in *Sch. pombe*. Now that the Gpa1 protein has been identified as a putative key transmitter of the signal in this pathway, it will be instructive to examine the direct or indirect interactions between this protein and the Ras protein.

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